AMENDMENT NO. 1 AUGUST 2017
TO
IS 13657 : 1993 ASPARTAME, FOOD GRADE — SPECIFICATION

[*Page* 1, *Table* 1, *Sl No*. (vii)] — Insert the following new entry at the end of the table:

Sl No.	Characteristic	Requirement	Method of Test, Ref to	
			Annex of this Standard	Other Standard
(1)	(2)	(3)	(4)	(5)
ix)	Other optical isomers	To pass the test	A-6	IS 1699

(*Page* 4, *clause* **A-5.2.2**) — Insert the following at the end:

‘A-6 TEST FOR OTHE OPTICAL ISOMERS

A-6.1 Apparatus

A-6.1.1 Amino acid analyzer which is equipped with the following

a) 550-mm x 9-mm (i.d.) column packed with approximately 50 g of strong cation exchange resin (Ion Exchange Resin No. 2613, or equivalent), 29-m x 0.5 mm (i.d.),

b) Reaction coil,

c) Ninhydrin supply, and

d) A photometer with an interference filter for 570 nm and selenium photocell detector.

Operating conditions may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions:

1) Column temperature : 55°C
2) Reaction coil temperature : 100°C
3) Eluant : *p*H 5.28 citrate buffer solution
4) Pressure of eluant : 8 – 10 kg/cm^2
5) Flow rate of eluant : 60 ml/h

6) Pressure of ninhydrin solution : 2-5 kg/cm^2
7) Flow rate of ninhydrin solution : 30 ml/h
8) Photometric detector: measuring wavelength: 570 nm
9) Recorder full scale: absorbance: 0-0.1

A-6.2 Reagents and Solutions

A-6.2.1 *Sodium Citrate*

A-6.2.2 *Citric Acid*

A-6.2.3 *Hydrochloric Acid*

A-6.2.4 *Benzyl Alcohol*

A-6.2.5 *Sodium Chloride*

A-6.2.6 *Sodium Acetate*

A-6.2.7 *Acetic Acid,* glacial.

A-6.2.8 *Methyl Cellosolve*

A-6.2.9 *Nitrogen Gas*

A-6.2.10 *Ninhydrin*

A-6.2.11 *Stannous Chloride*

A-6.2.12 *Reference Standard of L-alpha-aspartyl-D-phenylalanine Methyl Ester*

A-6.2.13 *Reference Standard of L-alpha-aspartyl-L-phenylalanine Methyl Ester*

A-6.2.14 *Citrate Buffer Solution* (*p*H 5.28) — Dissolve 34.3 g of sodium citrate in about 400 ml of water, add 7.5 ml of hydrochloric acid TS (35 percent) and 5 ml of benzyl alcohol, and add sufficient water to make 1 000 ml.

A-6.2.15 *Citrate Buffer Solution* (*p*H 2.2) — Dissolve 1.4 g of sodium citrate, 13.0 g of citric acid and 10.9 g of sodium chloride in about 400 ml of water and add sufficient water to make 1 000 ml.

A-6.2.16 *Ninhydrin solution* — Pour 140 ml of water into a 500 ml beaker, add 82.0 g of sodium acetate, stir to dissolve completely, add 25 ml of glacial acetic acid and dilute to 250 ml with water. Adjust the *p*H of the solution to 5.51 ± 0.03 with glacial acetic acid or sodium acetate (Acetate buffer solution). Pour 750 ml of methyl-cellosolve into a 1 000 ml brown glass bottle and add 250 ml of acetate buffer solution. Supply nitrogen gas through the solution, while mixing, dissolving 20 g of ninhydrin and then 0.38 g of stannous chloride ($SnCl_2 \cdot 2H_2O$) in the solution. Allow the solution to stand for at least 24 h before use.

A-6.3 Standard Preparation

Transfer 2.50 mg of L-alpha-aspartyl-Dphenylalanine methyl ester Reference Standard (Available from Ajinomoto Co. Inc., 1-5-8 Kyobashi, Chuo-ku, Tokyo 104, Japan.) into a 100 ml volumetric flask, dissolve and dilute to volume with water (Solution A). Transfer 250 mg of L-alpha-aspartyl-L-phenylalanine methyl ester Reference Standard into another 100-ml volumetric flask, dissolve in *p*H 2.2 citrate buffer solution, add 10.0 ml of Solution A and dilute to volume with *p*H 2.2 citrate buffer solution. Store this preparation below $5^{o}C$.

A-6.4 Sample Preparation

Transfer 250 mg of the sample into a 100 ml volumetric flask, dissolve and dilute to volume with *p*H 2.2 citrate buffer solution.

A-6.5 Procedure

A-6.5.1 Regenerate the column with 0.2 N sodium hydroxide TS and then buffer with *p*H 5.28 citrate buffer solution.

A-6.5.2 After conducting ninhydrin solution to the system, inject a 500 µl portion of the Standard Preparation into the amino acid analyzer and obtain the chromatogram.

A-6.5.3 Under the stated conditions, the retention time is about 100 min for L-alpha-aspartyl-D-phenylalanine methyl ester and about 115 min for L-alpha-aspartyl-L-phenylalanine methyl ester, respectively.

A-6.5.4 Similarly, inject a 500 µl portion of the sample preparation and obtain the chromatogram. Compare this chromatogram with that of the standard preparation and identify the component by comparing the retention time.

A-6.5.5 No peak corresponding to L-alpha-aspartyl-D-phenylalanine methyl ester and D-alpha-aspartyl-Lphenylalanine methyl ester shall be observed.

A-6.5.6 The detection limit for the sum of L-alpha-aspartyl-D-phenylalanine methyl ester and D-alpha-aspartyl-L-phenylalanine methyl ester in this method is about 1 µg/ml.'

(FAD 08)

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